

Fig 1. PCR cloning strategy for the genes encoding full length CCII

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107534538**COL2A1 cDNA corresponding to protein structure**

Signal peptide	N-Propeptide
MHGRPPRSAALLLLLLLLTAAAAA	
QDRDLRQPGPKGQKGE PGDIKDVVGPRGPGPQGPAGEQQQRGDRGEKGEKGAPGRGRDGEPTGPNPGPPGPPGPPGLGGN	
FAA QWAGGFDEKAGGAQMGVMQ GPMGPMGPRGPPGPTGAPGPQG	
N-Telopeptide	Helical domain
PQGNPGEPGEPGAAGPMGPR GPPGPPGKPGDDGETGKPKSGERGPPGPQGARGFPPTPLPGVKHRYGYPGLDGAKG	
EAGAPGAKGESCSPEGNGSPGPMGRGLPGERGRPGPSGAAGARGNDGLPGPAGPPGPVGPAGAPGFPAGPSKGEAGPTGARGPE	
GAQQPRGESGTPGSPGAPAGPNPGTDGIPGAKGSAGAPGIAGAPGFPGRGPPGPQATGTLGPKQGTGEPIAGFKGEQGPKE	
TGPAGPQGAPGAGEEKGARGEPGAAGPVGPPGERGAPGNRGFPQDGLAGPKGAPGERGPAGLAGPKGATGDPGRPGEPGLPG	
ARGLTGRPGDAGPQGVPTGAPGEDGRPGPPGPQARGQPCVMGFPGKANGEPGKAGEKGLPGAPGLRGLPGKDGETGAAGPP	
GPAGPVGERGEQAGPGPSGFQGLPGPPGPPGESGKPGDQGVPEAGAPLVGPRGERGFPGERGSPGAQGLQGRGLPGTPTDGP	
KGATGPAGPNGAQQPPGLQGMPPGERGAAGIAGLKGDRGDVGEKGPBAGPKDARGLTGPIGPPGAPNGEKGESGPPGPSGAAG	
ARGAPGERGEPGAPGAGFAGPPGADGQPGAKGEQGEPPGQKDGAPGPPGSPGAPGPGPTGVTGPKARGAQQPPGATGFPGAA	
GRVGPNGPNNGPPGPPGSAGKDGPKGYRGDAGPPGRAGDPGLQGPAGPPGEKGEPEGEDGPAGPDGPPGQGLAQGRGIVGLPGQ	
RGERGFPGLPGPSGEPGKQAGPSAGDRGPPGPVGPPLTGPAGEPGREGNPGADGLPGRDGAAGVKGDRGETGPVGAPGAPGAPG	
APGPVGPCTGKQDGRGETGAQGMGPPSGPAGARGMPGPQGRGDKGETGEAGERGLKGRGFTGLQGLPGPPGPSGDQGAAGPAGPS	
GPRGPPGPV	
	C-Telopeptide
GPSGKDGSNMGPIGPPGPRGRS GEPGPAGPPGNPGPPGPPGPP GTGIDMSAFAGLQTE KGPDPPIRYMRA DEA	
C-Propeptide	
AGGLRQHDVEVDATLKSNNQIESIRSPEGSKKNPARTCRDIKLCHPEWKSGDYWIDPNQGCTLDAIKVFCNMETGET	
CVYPTPSSIPRKNWTSKTKDKKHVWFAETINGGFHFSYGDENLSPNTASIQMTFLRLLSTEGSQNVTHCKNSIAYMDEETGNLK	
KAILIQGSNDVEIRAEGNSRPTYSVLEDGCTKHTGKWGKTIVYRLQKTSRLSIVDTAPMDIGGADQEFQVDIGPVCFL	

Fig 2: protein structure of chicken type II collegan

M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 M



Fig 3: Results from RT-PCR of 17-day-old chicken embryo CCOL2A1 3' UTR
1.heart, 2.liver, 3.vitreum, 4.cornea, 5.skin, 6.thymus gland, 7.pectoralis, 8.cornea, 9. small intestine, 10. arthroal cartilage, 11.spleen, 12. meniscus, 13.skull, 14.testis



Fig 4: Results from RT-PCR of adult chicken CCOL2A1 3' UTR
1.heart, 2.liver, 3.vitreum, 4.cornea, 5.skin, 6.thymus gland, 7.pectoralis, 8.cornea, 9. small intestine, 10. arthroal cartilage, 11.spleen, 12. meniscus, 13.skull, 14.testis

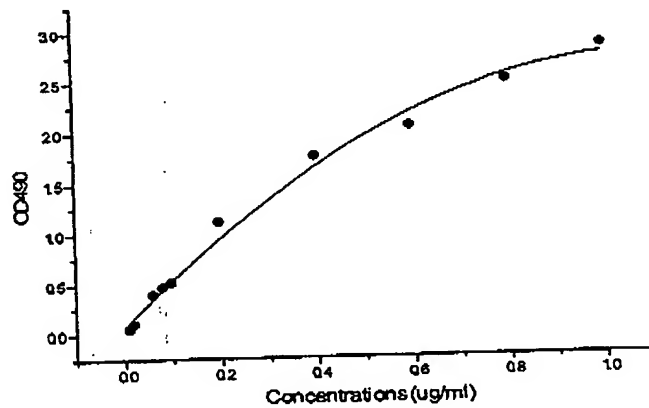


Fig5: standard curve of CCII concentration-absorbance

1 2 3 4 5 6 7 8 M



Fig 6: splicing analysis of COL2A1 exon 2
1. liver, 2. skin, 3. vitreum, 4. small intestine, 5. pectoralis,
6. cornea, 7. articular cartilage, 8. breast bone 9. M

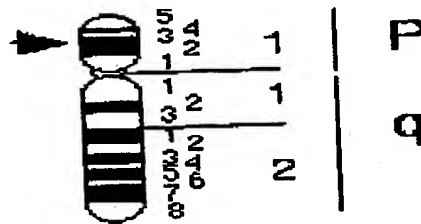


Fig.7: the chromosome band of the genes encoding CCII



Fig 8. division phase of the chicken chromosome

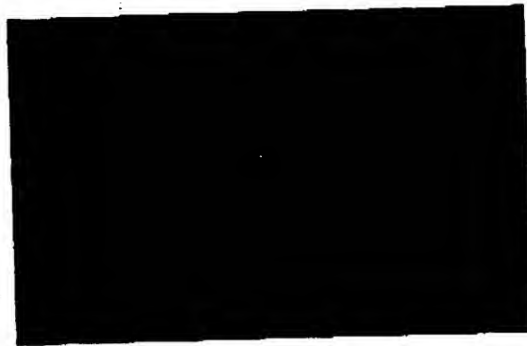


Fig 9 results of ISH hybridization

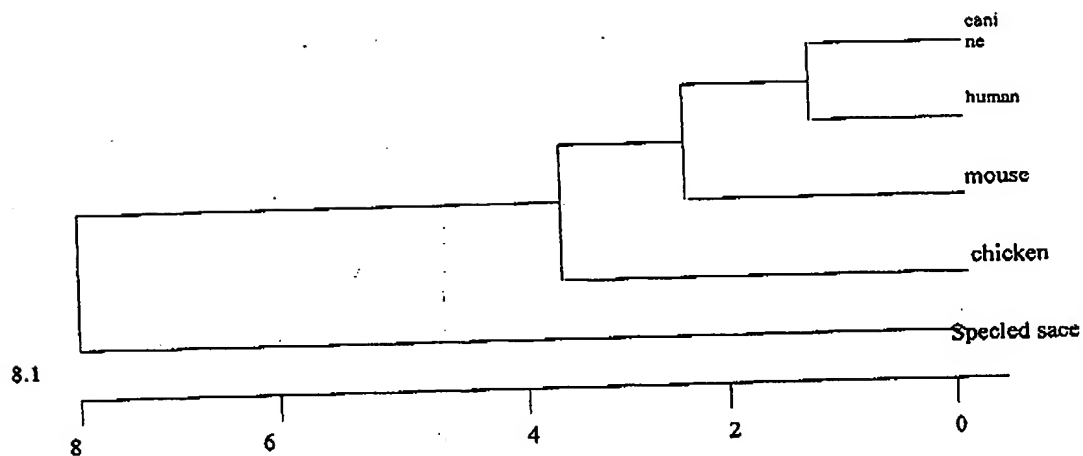


Fig.10: homologous comparison of the evolutionary tree between human being, dog, mouse, chicken and speckled dace

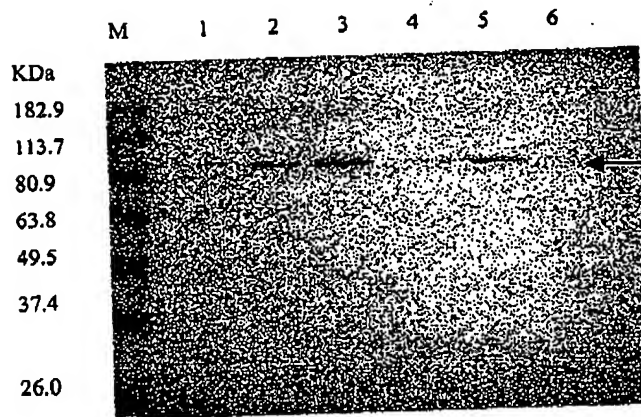


Fig. 11(A). Western blot analysis of pPIC9K/CCOL2A1 cytolysates.

M:

Lanes 1-5: bands for the induced cell lysis of pPIC9K/CCOL2A1. Lane 6: negative control transformed with vector DNA only. Protein molecular weight standard is shown at left (M).

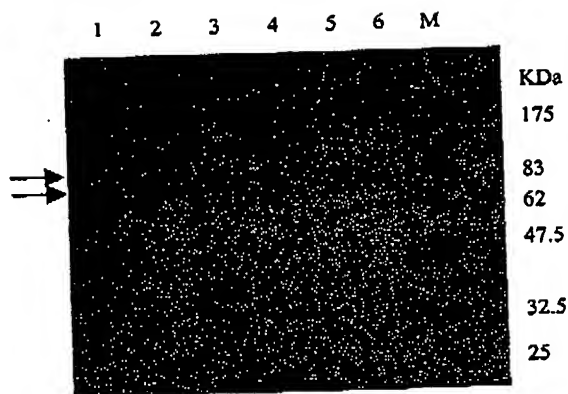


Fig 11(B). pPICZαB/CCOL2A1 cytolysate Western blot results. Protein molecular weight standard is shown at right (M).

10/534538
Rec'd PCT/PTO 12 MAY 2005

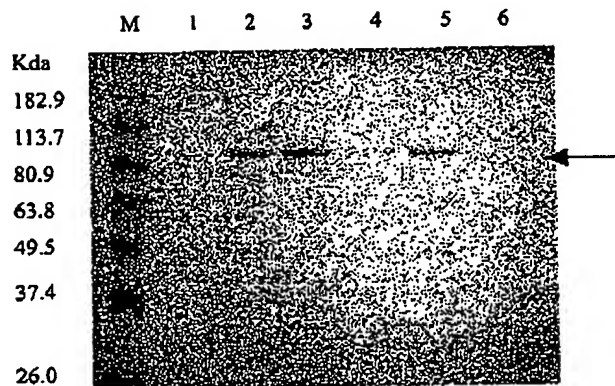


Fig. 12. Western blot analysis of the cell lysis CCOL2A1 produced by pPICZαB/CCOL2A1 co-expression with pPIC9K/P4Hα and pPIC9/P4H β

Lanes 1-5: positive bands for the induced cell lysis of CCOL2A1

Lane 6: negative control transformed with vector DNA only